Fully automated disc diffusion for rapid antibiotic susceptibility test results: a proof-of-principle study

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Abstract: Background Antibiotic resistance poses a significant threat to patients suffering from infectious diseases. Early readings of antibiotic susceptibility test (AST) results could be of critical importance to ensure adequate treatment. Disc diffusion is a well-standardized, established and cost-efficient AST procedure; however, its use in the clinical laboratory is hampered by the many manual steps involved, and an incubation time of 16-18 h, which is required to achieve reliable test results. Methods We have evaluated a fully automated system for its potential for early reading of disc diffusion diameters after 6-12 h of incubation. We assessed availability of results, methodological precision, categorical agreement and interpretation errors as compared with an 18 h standard. In total, 1028 clinical strains (291 Escherichia coli , 272 Klebsiella pneumoniae , 176 Staphylococcus aureus and 289 Staphylococcus epidermidis) were included in this study. Disc diffusion plates were streaked, incubated and imaged using the WASPLab TM automation system. Results and conclusions Our results demonstrate that: (i) early AST reading is possible for important pathogens; (ii) methodological precision is not hampered at early timepoints; and (iii) species-specific reading times must be selected. As inhibition zone diameters change over time and are phenotype/drug combination dependent, specific cut-offs and expert rules will be essential to ensure reliable interpretation and reporting of early susceptibility testing results.

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Fully automated disc diffusion for rapid antibiotic susceptibility test results: a proof-of-principle study

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Background: Antibiotic resistance poses a significant threat to patients suffering from infectious diseases. Early readings of antibiotic susceptibility test (AST) results could be of critical importance to ensure adequate treatment. Disc diffusion is a well-standardized, established and cost-efficient AST procedure; however, its use in the clinical laboratory is hampered by the many manual steps involved, and an incubation time of 16–18 h, which is required to achieve reliable test results.

Methods: We have evaluated a fully automated system for its potential for early reading of disc diffusion diameters after 6–12 h of incubation. We assessed availability of results, methodological precision, categorical agreement and interpretation errors as compared with an 18 h standard. In total, 1028 clinical strains (291 *Escherichia coli*, 272 *Klebsiella pneumoniae*, 176 *Staphylococcus aureus* and 289 *Staphylococcus epidermidis*) were included in this study. Disc diffusion plates were streaked, incubated and imaged using the WASPLab™ automation system.

Results and conclusions: Our results demonstrate that: (i) early AST reading is possible for important pathogens; (ii) methodological precision is not hampered at early timepoints; and (iii) species-specific reading times must be selected. As inhibition zone diameters change over time and are phenotype/drug combination dependent, specific cut-offs and expert rules will be essential to ensure reliable interpretation and reporting of early susceptibility testing results.

Introduction

Due to the continuous rise in antibiotic resistance, susceptibility patterns of bacterial infectious disease pathogens are becoming less predictable—a trend that is having a negative impact on patient healthcare.[1] Early and effective antibiotic treatment has been demonstrated to significantly improve clinical outcome and to reduce mortality.[2,3] The time required for conventional antibiotic susceptibility tests (ASTs) can result in a significant delay in the administration of an effective drug: the likelihood of antibiotic resistance to the empirical therapy selected is increasing and timely information on antibiotic susceptibility becomes of particular importance.[4] Other consequences of unknown antibiotic resistance are the use of more toxic agents or an unnecessary broad-spectrum therapy.[1] Rapid availability of accurate results from ASTs is currently considered one of the most important unmet medical needs in the management of infectious diseases.[5,6]

Automated microdilution ASTs provide results within 6–12 h but have a number of disadvantages, including fixed drug panels, low resolution (few drug concentrations tested), the need for a separate check for purity of culture, poor detection of synergism/antagonism phenomena, and comparably low sensitivity/specificity for important resistance mechanisms such as ESBLs, carbapenemases or inducible *erm*-mediated macrolide, lincosamide and streptogramin resistance (MLS).[7–9] Molecular detection of resistance determinants is rapid in principle, but hampered by the vast number of resistance mechanisms to cover. Molecular ASTs are, by nature, focused on specific genetic elements, making maintenance of accurate coverage, and hence detection of the most relevant resistance genes, a laborious task considering the different epidemiologies worldwide.[10] In addition, the presence of genes alone does not necessarily correlate with expression and phenotypic resistance.

Resistance detection by MALDI-TOF has also been described, but is limited to specific targets such as PBP2a, ESBLs or carbapenemases.[11–14] Microfluidic systems have recently been described as a potential tool for performing rapid ASTs within 6 h from blood culture broth.[15] Both techniques, however, are still in their infancy, and currently not designed for high-throughput ASTs.

Disc diffusion is still an affordable, accurate, reliable and highly standardized AST method with the advantages of low consumable costs, flexible drug testing and recognition of additional phenomena such as synergisms for the detection of ESBLs and/or antagonisms for the detection of *erm*MLS or AmpCs.[16–19] However, the

standard incubation time recommended in CLSI and EUCAST guidelines is 16–18 h for most pathogens.[20,21]

This study aimed at analysing the technical feasibility of a rapid disc diffusion AST (rAST), comprising early disc diffusion zone diameter reading at 6–12 h using the fully automated WASPLab™ system (Copan Italia).[22] The study focused on the utility of earlier (<18 h) readings, the influence of early reading on precision/reproducibility and the influence of early reading on categorical agreement with EUCAST 18 h clinical breakpoints (CBPs) for pathogens that are most prevalent in positive blood cultures/sepsis.

Methods

Quality control (QC) strains

For testing methodological precision and accuracy, 59 repetitive disc diffusion ASTs of *Escherichia coli* ATCC 25922 and 58 repetitive disc diffusion ASTs of *Staphylococcus aureus* ATCC 29213 EUCAST QC strains were done from individual fresh subcultures and individually prepared 0.5 McFarland standards. Interpretation was done according to EUCAST QC tables version 6.1.[23]

Clinical isolates

Study isolates were selected to cover a broad range of inhibition zone diameters for each species/drug combination tested (see Figure S1, available as Supplementary data at *JAC* Online). In particular, critical isolates close to the CBPs were included. All non-duplicate clinical strains included in this study were isolated over a 3 year period from 2013 to 2016 in the clinical microbiology laboratory of the Institute of Medical Microbiology, University of Zurich. Isolates of the same species were considered duplicate(s) if they: (i) originated from the same patient; and (ii) showed no more that one major *and* two minor differences in AST interpretation. The following numbers of clinical isolates were tested: *E. coli* ($n = 291$), *Klebsiella pneumoniae* ($n = 272$), *S. aureus* ($n = 176$) and *Staphylococcus epidermidis* ($n = 289$).

Susceptibility testing

Susceptibility testing and clinical categorization was performed according to EUCAST guidelines version 6.0, which are essentially the same standards as CLSI 2016.[20,21] In brief, bacterial suspensions were manually adjusted to a turbidity equivalent to that of a 0.5 McFarland standard and processed within 15 min. Mueller–Hinton II agar plates (Oxoid Ltd, Basingstoke, UK) were processed in the fully automated WASP™ system (Copan Italia SpA, Brescia, Italy), i.e. plates were each inoculated with 60 μL of the bacterial suspension and automatically streaked. Antibiotic discs of a single production lot (Oxoid) were placed using a standard distributor, which was handled by the WASP™ robot immediately after plate streaking. Subsequently, plates were automatically transported to and incubated in a WASPLab™ incubator (Copan) at 37 ± 2°C in ambient air. Images were taken after 6, 8, 12 and 18 h of incubation under continuous temperature conditions. Diameter measurements were automatically done by the WASPLab™ reading software (Copan) and were, if necessary, adjusted on-screen by an experienced technician.

Statistical analysis and software

All statistical analyses were performed using R, version 3.2.3.[24] For the QC strains, significance of deviations from target values issued by EUCAST was assessed using one-sample *t*-tests with the Bonferroni correction ($\alpha = 0.05$). Linear mixed models were used to model the influence of reading time on reading precision. The antibiotic was treated as a random effect and the R package nlme, version 3.1-128, was used.[25] For *S. aureus* ATCC 29213 a paired *t*-test was used to test whether precision at 6 h was significantly different from the mean precision at later reading times. For the clinical isolates, readability and categorical agreement with reading after 18 h were analysed using logistic regression with reading time, species and antibiotic as predictors. Significance of coefficients was assessed using likelihood-ratio tests.

Results

Methodological precision and accuracy

The methodological precision of the disc diffusion AST was assessed using EUCAST QC strains *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 and the following antibiotics: *E. coli*: ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefuroxime, cefoxitin, cefpodoxime, ceftriaxone, cefepime, meropenem, norfloxacin, ciprofloxacin, levofloxacin, amikacin, gentamicin, tobramycin, tigecycline, nitrofurantoin and trimethoprim/sulfamethoxazole; *S. aureus*: penicillin G, cefoxitin, norfloxacin, ciprofloxacin, levofloxacin, gentamicin, tobramycin, clindamycin, erythromycin, tetracycline, minocycline, tigecycline, linezolid, fusidic acid, rifampicin and trimethoprim/sulfamethoxazole. The 59 repetitions for *E. coli* ATCC 25922 and the 58 repetitions for *S. aureus* ATCC 29213 each read at 6, 8, 12 and 18 h resulted in a total of 4248 and 3712 data points, respectively. All 18 h values were in full agreement with EUCAST QC requirements as reflected by measuring variation ranges in this study, generally displaying half of the variation of the accepted EUCAST QC range or less (Table 1).[23]

The methodological precision of early reading was within ±0.2 mm of that of the 18 h standard incubation time except for the *S. aureus* ATCC 29213 6 h reading: the average 1-fold standard deviation of all drugs tested at 18 h was 0.9 mm for *E. coli* ATCC 25922 and 0.7, 0.7 and 0.8 mm for the 6, 8 and 12 h readings, respectively; the observed increase of standard deviation over time was thus small (0.2 mm), but was statistically significant ($P = 0.003$; Table 1). The 1-fold standard deviation of all drugs tested at 18 h was 1.1 mm for *S. aureus* ATCC 29213 and 5.1, 1.2 and 1.2 mm for the 6, 8 and 12 h readings, respectively. The standard deviation was significantly higher at 6 h as compared with later reading times ($P = 1 \times 10^{-07}$) and no statistical evidence for systematic change in precision for later reading times was found ($P = 0.08$; Table 1).

In addition, we assessed calibration of the test system to given EUCAST targets: at 18 h of incubation, the mean diameter values of 11 out of 18 drugs and *E. coli* ATCC 25922 matched EUCAST target values or deviated by ≤1 mm (81.0%); for 7 drugs (19%) the mean diameter values deviated 2 mm from the target (Table 1).[26] For *S. aureus* ATCC 29213 6 out of 16 drugs (37.5%) deviated 0–1 mm from the EUCAST target, 6 drugs deviated 2 mm from target and for 4 drugs (penicillin G, tobramycin, tetracycline and tigecycline) the mean diameter values deviated 3 mm from the EUCAST target.

Readability

Readability was defined as the percentage of data points for which a diameter measurement could reliably be determined. The following antibiotics were tested for Enterobacteriaceae and read at 6, 8, 12 and 18 h: ampicillin (*E. coli* only), amoxicillin/clavulanate, piperacillin/tazobactam, cefuroxime, cefoxitin, cefpodoxime,

Table 1. Methodological precision and agreement with EUCAST QC ranges of disc diffusion zone diameter measurements at 18, 12, 8 and 6 h of incubation for QC strains *E. coli* ATCC 25922 and *S. aureus* ATCC 29213

	Zone diameter values (mm)															
	E. coli ATCC 25922								*S. aureus* ATCC 29213							
					range		range width						range		range width	
Incubation time/drug	mean study	target EUCAST 18 h	Δmean −target 18 h	SD	EUCAST QC	study	EUCAST QC	study	mean study	target EUCAST 18 h	Δmean −target 18 h	SD	EUCAST QC	study	EUCAST QC	study
18 h																
penicillin G									12	15	−3	1.1	12–18	10–15	6	5
ampicillin	17	19	−2	1.2	16–22	15–20	6	5								
amoxicillin/clavulanate	20	21	−1	0.5	18–24	19–21	6	2								
piperacillin/tazobactam	22	24	−2	0.6	21–27	21–23	6	2								
cefuroxime	21	23	−2	0.5	20–26	20–22	6	2								
cefoxitin	25	26	−1	1.0	23–29	23–27	6	4	29	27	2	0.9	24–30	27–31	6	4
cefpodoxime	24	26	−2	0.6	23–28	23–25	5	2								
ceftriaxone	30	32	−2	0.9	29–35	28–32	6	4								
cefepime	32	34	−2	1.0	31–37	30–34	6	4								
meropenem	31	31	0	1.1	28–34	28–33	6	5								
norfloxacin	31	32	−1	1.0	28–35	29–33	7	4	22	21	1	0.9	18–24	20–23	6	3
ciprofloxacin	34	35	−1	0.9	30–40	32–36	10	4	23	24	−1	1.0	21–27	21–25	6	4
levofloxacin	32	33	−1	1.1	29–37	30–34	8	4	24	26	−2	1.1	23–29	22–27	6	5
amikacin	24	23	1	1.1	19–26	22–26	7	4								
gentamicin	23	23	0	0.8	19–26	21–24	7	3	20	22	−2	0.7	19–25	18–21	6	3
tobramycin	21	22	−1	0.8	18–26	20–23	8	3	20	23	−3	0.7	20–26	19–22	6	3
clindamycin									25	26	−1	0.9	23–29	23–26	6	3
erythromycin									24	26	−2	1.1	23–29	22–26	6	4
tetracycline									24	27	−3	1.1	23–31	22–26	8	4
minocycline									24	26	−2	1.2	23–29	22–26	6	4
tigecycline	22	24	−2	0.7	20–27	20–23	7	3	19	22	−3	0.9	19–25	18–21	6	3
linezolid									23	24	−1	1.1	21–27	20–25	6	5
fusidic acid									27	29	−2	1.6	26–32	24–30	6	6
nitrofurantoin	20	20	0	0.6	17–23	18–21	6	3								
rifampicin									32	33	−1	1.7	30–36	28–35	6	7
trimethoprim/sulfamethoxazole	26	26	0	0.7	23–29	24–27	6	3	29	29	0	1.0	26–32	27–31	6	4
average			−1.0	0.9			6.7	3.6			−1.4	1.1			6.1	4.2
12 h																
penicillin G									13	NA	−2	1.2	NA	10–15	6	5
ampicillin	17	NA	−2	0.9	NA	15–19	6	4								
amoxicillin/clavulanate	20	NA	−1	0.4	NA	19–21	6	2								
piperacillin/tazobactam	22	NA	−2	0.6	NA	21–23	6	2								

Continued

Table 1. *Continued*

	Zone diameter values (mm)															
	E. coli ATCC 25922								*S. aureus* ATCC 29213							
					range		range width						range		range width	
Incubation time/drug	mean study	target EUCAST 18 h	Δmean −target 18 h	SD	EUCAST QC	study	EUCAST QC	study	mean study	target EUCAST 18 h	Δmean −target 18 h	SD	EUCAST QC	study	EUCAST QC	study
cefuroxime	20	NA	−3	0.5	NA	19–21	6	2								
cefoxitin	24	NA	−2	0.8	NA	23–26	6	3	28	NA	1	0.8	NA	27–30	6	3
cefpodoxime	23	NA	−3	0.6	NA	22–25	5	3								
ceftriaxone	29	NA	−3	0.9	NA	27–31	6	4								
cefepime	31	NA	−3	1.0	NA	29–33	6	4								
meropenem	30	NA	−1	1.1	NA	28–32	6	4								
norfloxacin	31	NA	−1	0.9	NA	29–33	7	4	21	NA	0	0.8	NA	20–23	6	3
ciprofloxacin	34	NA	−1	0.9	NA	32–35	10	3	23	NA	−1	1.1	NA	20–25	6	5
levofloxacin	32	NA	−1	0.9	NA	30–34	8	4	24	NA	−2	1.1	NA	22–27	6	5
amikacin	23	NA	0	0.7	NA	21–24	7	3								
gentamicin	22	NA	−1	0.6	NA	21–23	7	2	20	NA	−2	0.7	NA	18–21	6	3
tobramycin	20	NA	−2	0.5	NA	19–21	8	2	20	NA	−3	0.7	NA	19–21	6	2
clindamycin									24	NA	−2	1.4	NA	21–27	6	6
erythromycin									24	NA	−2	1.2	NA	22–26	6	4
tetracycline									24	NA	−3	1.4	NA	21–27	8	6
minocycline									24	NA	−2	1.4	NA	21–26	6	5
tigecycline	22	NA	−2	0.8	NA	20–23	7	3	19	NA	−3	1.1	NA	17–22	6	5
linezolid									24	NA	0	1.6	NA	21–27	6	6
fusidic acid									27	NA	−2	2	NA	23–31	6	8
nitrofurantoin	20	NA	0	0.7	NA	19–22	6	3								
rifampicin					NA				32	NA	−1	1.2	NA	30–34	6	4
trimethoprim/sulfamethoxazole	26	NA	0	0.6	NA	25–27	6	2	28	NA	−1	1.2	NA	26–31	6	5
average			−1.3	0.8			6.7	3.3			−1.6	1.2			6.1	4.7
8 h																
penicillin G									13	NA	−2	1	NA	11–15	6	4
ampicillin	17	NA	−2	0.4	NA	16–18	6	2								
amoxicillin/clavulanate	20	NA	−1	0.4	NA	19–20	6	1								
piperacillin/tazobactam	21	NA	−3	0.6	NA	20–23	6	3								
cefuroxime	20	NA	−3	0.5	NA	19–21	6	2								
cefoxitin	24	NA	−2	0.4	NA	23–25	6	2	24	NA	−3	0.7	NA	23–26	6	3
cefpodoxime	23	NA	−3	0.6	NA	22–24	5	2								
ceftriaxone	28	NA	−4	0.9	NA	26–30	6	4								
cefepime	30	NA	−4	1.0	NA	28–32	6	4								
meropenem	29	NA	−2	1.3	NA	27–32	6	5								
norfloxacin	29	NA	−3	0.6	NA	28–31	7	3	19	NA	−2	0.7	NA	18–21	6	3
ciprofloxacin	32	NA	−3	0.7	NA	31–34	10	3	21	NA	−3	0.9	NA	19–23	6	4

levofloxacin	30	NA	−3	0.6	NA	29–32	8	3	23	NA	−3	0.9	NA	22–25	6	3
amikacin	21	NA	−2	0.4	NA	20–21	7	1								
gentamicin	20	NA	−3	0.4	NA	19–21	7	2	19	NA	−3	0.8	NA	17–21	6	4
tobramycin	19	NA	−3	0.3	NA	19–20	8	1	20	NA	−3	0.6	NA	18–21	6	3
clindamycin									24	NA	−2	1.9	NA	20–27	6	7
erythromycin									23	NA	−3	1.5	NA	20–26	6	6
tetracycline									23	NA	−4	1.4	NA	20–26	8	6
minocycline									23	NA	−3	1.2	NA	20–25	6	5
tigecycline	21	NA	−3	1.0	NA	19–23	7	4	18	NA	−4	1.7	NA	15–21	6	6
linezolid									24	NA	0	1.8	NA	21–28	6	7
fusidic acid									26	NA	−3	2	NA	22–30	6	7
nitrofurantoin	21	NA	1	0.7	NA	20–23	6	3								
rifampicin									31	NA	−2	0.8	NA	29–32	6	3
trimethoprim/sulfamethoxazole	26	NA	0	0.7	NA	25–28	6	3	26	NA	−3	1.6	NA	23–29	6	6
average			−1.7	0.7			6.7	3.0			−2.7	1.2			6.1	4.8
6 h																
penicillin G									12	NA	−3	2.2	NA	8–17	6	9
ampicillin	17	NA	−2	0.4	NA	16–18	6	2								
amoxicillin/clavulanate	19	NA	−2	0.4	NA	18–20	6	2								
piperacillin/tazobactam	21	NA	−3	0.5	NA	20–22	6	2								
cefuroxime	20	NA	−3	0.5	NA	19–21	6	2								
cefoxitin	22	NA	−4	0.4	NA	21–23	6	2	20	NA	−7	4.3	NA	12–29	6	17
cefpodoxime	22	NA	−4	0.6	NA	21–23	5	2								
ceftriaxone	27	NA	−5	0.9	NA	25–29	6	4								
cefepime	29	NA	−5	0.9	NA	27–31	6	4								
meropenem	28	NA	−3	0.9	NA	26–30	6	4								
norfloxacin	27	NA	−5	0.5	NA	26–28	7	2	16	NA	−5	3.1	NA	10–22	6	12
ciprofloxacin	30	NA	−5	0.5	NA	29–31	10	2	17	NA	−7	3.5	NA	10–24	6	14
levofloxacin	28	NA	−5	0.5	NA	27–29	8	2	19	NA	−7	4.1	NA	11–28	6	17
amikacin	19	NA	−4	0.5	NA	18–20	7	2								
gentamicin	19	NA	−4	0.4	NA	18–20	7	2	17	NA	−5	3.3	NA	10–24	6	12
tobramycin	18	NA	−4	0.4	NA	17–19	8	2	17	NA	−6	3.8	NA	10–25	6	15
clindamycin									20	NA	−6	5.8	NA	9–32	6	23
tetracycline									19	NA	−8	6.2	NA	6–31	8	8
minocycline									16	NA	−10	8	NA	0–32	6	32
tigecycline	20	NA	−4	0.9	NA	18–22	7	4	13	NA	−9	5.6	NA	2–24	6	22
linezolid									22	NA	−2	4.9	NA	12–31	6	19
fusidic acid									22	NA	−7	6.3	NA	10–35	6	25
nitrofurantoin	23	NA	3	1.3	NA	20–26	6	6		NA			NA			
rifampicin									22		−11	9.1		3–40	6	37
trimethoprim/sulfamethoxazole	26	NA	0	0.6	NA	25–27	6	2	20	NA	−9	4.8	NA	10–29	6	19
average			−2.6	0.7			6.7	2.9			−6.8	5.1			6.1	19.0

NA, not applicable.

Data represent 59 repetitive measurements of *E. coli* ATCC 25922 and 58 repetitive measurements of *S. aureus* ATCC 29213 from individual fresh subcultures and individually prepared 0.5 McFarland standards. Interpretation followed EUCAST QC tables version 6.1. Deviations of early reading times from EUCAST 18 h target values were statistically significant at the significance level $\alpha = 0.05$ in all species/drug combinations and reading times except for *S. aureus* and linezolid at 6 h applying one-sample *t*-tests with the Bonferroni correction.

ceftriaxone, cefepime, meropenem, norfloxacin, ciprofloxacin, levofloxacin, amikacin, gentamicin, tobramycin, tigecycline, nitrofurantoin (*E. coli* only) and trimethoprim/sulfamethoxazole. The following antibiotics were tested for staphylococci and read at 6, 8, 12 and 18 h: penicillin G (*S. aureus* only), cefoxitin, norfloxacin, ciprofloxacin, levofloxacin, gentamicin, tobramycin, clindamycin, erythromycin, tetracycline, minocycline, tigecycline, linezolid, fusidic acid, rifampicin and trimethoprim/sulfamethoxazole, resulting in a total of 66 964 data points, i.e. 20 952, 17 408, 11 264 and 17 340 data points for *E. coli* ($n = 291$), *K. pneumoniae* ($n = 272$), *S. aureus* ($n = 176$) and *S. epidermidis* ($n = 289$), respectively.

Logistic regression was used to model observed readabilities and a significant increase in readability over time was found ($P < 2 \times 10^{-16}$). In addition, significant differences between species were observed ($P < 2 \times 10^{-16}$). Average readability at early timepoints was, in part, higher for *E. coli* and *K. pneumoniae* than for *S. aureus* and *S. epidermidis* (99.4%, 99.0%, 82.2% and 19.8% at 6 h, respectively; 100%, 99.6%, 97.9%, 97.9% and 63.8% at 8 h, respectively; and 100%, 100%, 100% and 99.4% at 12 h, respectively; Table 2). While there were only minor variations between readability of individual drugs for the Enterobacteriaceae, readability of different drugs for staphylococci ranged from 62.5% for tetracycline to 96.6% for norfloxacin (*S. aureus* at 6 h; Table 2) and from 10.7% for tetracycline to 41.2% for erythromycin and clindamycin (*S. epidermidis* at 6 h).

Categorical agreement

Categorical agreement of early readings increased significantly over time when EUCAST 18 h CBPs were applied ($P < 2 \times 10^{-16}$): the average agreement for clinical strains and all drugs tested at 6 h was 93.5%, 93.3%, 48.7% and 77.5% for *E. coli*, *K. pneumoniae*, *S. aureus* and *S. epidermidis*, respectively, and increased to 96.6%, 95.9%, 88.8% and 89.3% at 8 h and to 98.7%, 98.4%, 99.0% and 97.2% at 12 h (Table 2).

Significant differences were observed between species ($P < 2 \times 10^{-16}$) and between individual drugs ($P < 2 \times 10^{-16}$), e.g. categorical agreement at 6 h varied from 82.3% for trimethoprim/sulfamethoxazole to 99.7% for ampicillin and meropenem in *E. coli*, from 13.8% for minocycline to 97.8% for trimethoprim/sulfamethoxazole in *S. aureus*, and from 66.7% to 96.6% for levofloxacin and norfloxacin for the quinolones in *S. aureus* at 8 h (Table 2).

Change of zone diameters over time and interpretation errors

The majority of inhibition zone diameter values changed over time (see examples in Figure 1 and change patterns in Table 2). Decreasing, increasing and stable zone diameter patterns were observed for all species/drug combinations (Table 2). Most frequently, different diameter change patterns were observed in one and the same species/drug combination (see examples in Figure 1) and no clear correlation of a diameter change pattern and a specific drug or drug class was detected (Table 2).

Changes of zone diameters would result in interpretation errors at early reading times when applying EUCAST 18 h CBPs: increasing diameters were the most frequent pattern and resulted in major errors or minor errors depending on the relative position of the CBP, e.g. 70.1% major errors for cefoxitin and *S. aureus* at 6 h, and 12.9% minor errors for *E. coli* and norfloxacin at 6 h (Table 2 and Figure 1). Decreasing diameters resulted in very major errors, e.g. 11.1% very major errors for trimethoprim/sulfamethoxazole and *E. coli* at 6 h (Table 2).

Discussion

Automation of disc diffusion has been demonstrated to significantly improve standardization and to reduce manual workload.[27–29] In addition to offering improved standardization, this study demonstrates that automated disc diffusion in principle allows for early reading for the most important pathogens isolated from positive blood cultures, e.g. *E. coli*, *K. pneumoniae*, *S. aureus* and *S. epidermidis* accounted for 39.6% of all blood culture isolates in our laboratory in 2015. Most importantly, early reading did not impair methodological precision (Table 1). As zone diameters were adjusted on-screen an investigator-bias to better match QC requirements is theoretically possible. However, technicians did not have any information on the appropriate QC ranges next to them during zone reading. It seems unlikely that a person will be able to recall the high number of QC ranges and use this information to intentionally bias results.

The optimal early reading timepoints varied according to the species studied. The vast majority of zone diameters of *E. coli* and *K. pneumoniae* were readable after 6 h of incubation, while reliable reading for *S. aureus* was possible after 8 h and sufficient readability for *S. epidermidis* zones was achieved after 12 h of incubation (Table 2). Therefore, early reading times need to be adjusted to the species being analysed.

Zone diameters changed over time, leading to both major errors (false-resistant results) and very major errors (false-susceptible results) if CLSI- and EUCAST-recommended CBPs for 18 h incubation were applied (Table 2 and Figure 1). The patterns of diameter changes varied from decreasing diameters over stable zones to increasing diameters, and the change patterns were, in part, species/drug combination dependent. For the majority of species/drug combinations a mixture of diameter change patterns was found. These different patterns are most probably related to different phenotype entities, e.g. WT isolates and different non-WT populations. A specific analysis of the interdependence of resistance mechanisms and diameter change patterns is beyond the scope of this study, but will be essential for developing a reliable interpretation system for disc diffusion reading at early timepoints.

As existing CBPs of EUCAST and CLSI cannot be used to categorize zone diameters that are read at early timepoints, specific cut-offs for rAST must be used. Three settings can be distinguished that influence these time-dependent cut-offs (TDCs). (i) If diameter values are stable over time and/or no category changes occur over time for all WT and non-WT populations of a given species/drug combination, existing CLSI/EUCAST CBPs could readily be used as few interpretation errors occur, e.g. for ceftriaxone and *E. coli* (see Figure 1a). (ii) If zone diameters change over time and category changes occur, but susceptible and resistant populations can be discriminated at early reading times, TDCs may be set based on WT/non-WT populations as is done by EUCAST for the standard system using epidemiological cut-off values (ECOFFs). At 6, 8 or 12 h, ECOFFs could be determined and used as putative early CBPs, e.g. for *S. aureus* and cefoxitin (Figure 1c). (iii) If zone diameters change over time and resistant populations cannot be

Table 2. Early reading of disc diffusion susceptibility tests with clinical strains of *E. coli* ($n = 291$), *K. pneumoniae* ($n = 272$), *S. aureus* ($n = 176$) and *S. epidermidis* ($n = 289$) after 6, 8 and 12 h as compared with standard incubation at 18 h

	Zone diameter measurements and related classification parameters (all values in %)															
	6 versus 18 h					8 versus 18 h					12 versus 18 h					
Species/drug	readability	categorical agreement	vMEs	MEs	mEs	readability	categorical agreement	vMEs	MEs	mEs	readability	categorical agreement	vMEs	MEs	mEs	Diameter change patterns
E. coli, $n = 291$																
ampicillin	99.7	99.7	0.3	0.0	0.0	100	100	0.3	0.0	0.0	100	100	0.3	0.0	0.0	↑↓↔
amoxicillin/clavulanate	99.3	82.3	0.3	**17.4**	0.0	100	89.0	0.7	**10.7**	0.0	100	96.6	0.3	**3.4**	0.0	↑↓↔
piperacillin/tazobactam	99.3	92.0	0.0	0.3	**7.6**	100	96.6	0.3	0.0	3.4	100	99.3	0.0	0.0	1.0	↑↓↔
cefuroxime	99.3	96.9	**2.8**	0.3	0.0	100	97.6	**2.8**	0.0	0.0	100	97.9	**2.4**	0.0	0.0	↑↓↔
cefoxitin	99.7	93.1	**2.8**	**4.2**	0.0	100	95.2	**3.8**	**1.4**	0.0	100	99.3	**1.0**	0.0	0.0	↑↓↔
cefpodoxime	99.7	93.4	**1.4**	**5.2**	0.0	100	97.2	**2.4**	0.7	0.0	100	97.6	**2.1**	0.7	0.0	↑↓↔
ceftriaxone	99.7	99.3	0.0	0.0	0.7	100	100	0.0	0.0	0.3	100	100	0.0	0.0	0.3	↑↓↔
cefepime	99.3	97.6	0.0	0.0	2.4	100	99.0	0.0	0.0	1.4	100	99.3	0.0	0.0	1.0	↑↓↔
meropenem	99.7	99.7	0.0	0.0	0.3	100	100	0.0	0.0	0.3	100	100.3	0.0	0.0	0.0	↑↓↔
norfloxacin	98.6	87.1	0.0	0.0	**12.9**	100	93.8	0.0	0.0	**6.6**	100	99.0	0.0	0.0	1.4	↑↓
ciprofloxacin	99.0	93.7	0.0	0.0	**6.3**	100	97.6	0.0	0.0	2.8	100	100	0.0	0.0	0.3	↑↓↔
levofloxacin	99.0	88.5	0.0	0.3	**11.5**	100	95.2	0.0	0.0	**5.2**	100	99.7	0.0	0.0	0.7	↑↓
amikacin	99.3	96.9	0.0	0.0	3.1	100	97.6	0.0	0.0	2.8	100	99.0	0.3	0.0	1.0	↑↓↔
gentamicin	99.7	99.0	0.0	0.0	1.0	100	99.3	0.0	0.0	1.0	100	99.7	0.0	0.0	0.7	↑↓
tobramycin	99.0	87.5	0.0	0.0	**12.5**	100	90.0	0.0	0.0	**10.3**	100	94.5	0.0	0.0	**5.9**	↑↓↔
tigecycline	99.7	95.8	0.0	0.0	4.2	100	99.3	0.0	0.0	1.0	100	99.7	0.0	0.0	0.7	↑
nitrofurantoin	100	97.9	**1.0**	**1.0**	0.0	100	99.3	**1.0**	0.0	0.0	100	99.7	0.7	0.0	0.0	↑↓
trimethoprim/sulfamethoxazole	99.3	82.3	**11.1**	0.0	**6.6**	100	91.4	**3.8**	0.0	**5.2**	100	95.5	0.7	0.0	4.1	↑↓↔
average	99.4	93.5	1.1	1.6	3.8	100	96.6	0.8	0.7	2.2	100	98.7	0.4	0.2	1.0	
K. pneumoniae, $n = 272$																
amoxicillin/clavulanate	99.3	94.1	0.0	**5.9**	0.0	99.6	95.9	0.0	**4.1**	0.0	100	98.9	0.0	**1.1**	0.0	↑↓↔
piperacillin/tazobactam	98.9	86.2	0.0	0.0	**13.8**	99.3	92.2	0.0	0.0	7.8	100	97.4	0.0	0.0	2.6	↑↓↔
cefuroxime	98.9	95.9	**1.5**	**2.6**	0.0	99.3	97.0	**1.5**	**1.5**	0.0	100	98.9	**1.1**	0.0	0.0	↑↓↔
cefoxitin	98.9	94.8	**3.7**	**1.5**	0.0	100	96.7	**1.8**	**1.5**	0.0	100	98.9	0.7	0.4	0.0	↑↓↔
cefpodoxime	98.9	97.4	0.0	**2.6**	0.0	99.6	98.5	0.4	**1.1**	0.0	100	100	0.0	0.0	0.0	↑↓↔
ceftriaxone	99.3	98.5	0.4	0.0	1.1	99.6	99.6	0.0	0.0	0.4	100	99.6	0.0	0.0	0.4	↑↓↔
cefepime	98.9	97.8	0.0	0.0	2.2	99.6	99.6	0.0	0.0	0.4	100	100	0.0	0.0	0.0	↑↓↔
meropenem	99.3	98.1	0.0	0.0	1.9	99.6	98.9	0.0	0.0	1.1	100	99.6	0.0	0.0	0.4	↑↓↔
norfloxacin	98.5	90.3	0.4	0.0	**9.3**	99.3	94.8	0.0	0.0	**5.2**	100	98.5	0.0	0.0	1.5	↑↓↔
ciprofloxacin	98.9	91.8	0.0	0.4	**7.8**	99.6	93.7	0.0	0.4	**5.9**	100	98.5	0.0	0.0	1.5	↑↓↔
levofloxacin	98.9	88.8	0.0	0.0	**11.2**	99.6	94.5	0.0	0.0	**5.5**	100	97.1	0.0	0.0	2.9	↑↓↔
amikacin	99.3	97.0	0.0	0.0	3.0	99.6	98.5	0.0	0.0	1.5	100	99.3	0.0	0.0	0.7	↑↓↔
gentamicin	99.3	98.1	0.4	0.0	1.5	99.6	98.2	0.4	0.0	1.5	100	99.3	0.4	0.0	0.4	↑↓↔
tobramycin	98.9	95.5	0.4	0.0	4.1	99.6	97.0	0.4	0.0	2.6	100	99.6	0.4	0.0	0.0	↑↓↔
tigecycline	98.9	78.8	0.0	0.0	**21.2**	99.6	85.2	0.0	0.0	**14.8**	100	93.8	0.0	0.0	**6.3**	↑↔
trimethoprim/sulfamethoxazole	98.9	90.0	**6.3**	0.0	3.7	99.3	93.7	**2.6**	0.4	3.3	100	95.6	**1.1**	0.0	3.3	↑↓↔
average	99.0	93.3	0.8	0.8	5.0	99.6	95.9	0.4	0.6	3.1	100	98.4	0.2	0.1	1.2	

Continued

Table 2. *Continued*

Species/drug	Zone diameter measurements and related classification parameters (all values in %)															Diameter change patterns
	6 versus 18 h					8 versus 18 h					12 versus 18 h					
	readability	categorical agreement	vMEs	MEs	mEs	readability	categorical agreement	vMEs	MEs	mEs	readability	categorical agreement	vMEs	MEs	mEs	
S. aureus, $n = 176$																
penicillin g	92.0	77.8	0.0	**22.2**	0.0	98.3	78.6	0.0	**21.4**	0.0	100	99.4	0.0	0.6	0.0	↑↓↔
cefoxitin	94.9	29.9	0.0	**70.1**	0.0	98.3	99.4	0.0	0.6	0.0	100	100	0.0	0.0	0.0	↑↓↔
norfloxacin	96.6	41.8	0.0	**58.2**	0.0	98.9	96.6	0.0	0.0	3.4	100	99.4	0.0	0.6	0.0	↑↓↔
ciprofloxacin	92.0	29.0	0.0	**71.0**	0.0	98.9	68.4	0.0	**31.6**	0.0	100	94.3	0.0	**5.7**	0.0	↑↓↔
levofloxacin	91.5	19.3	0.0	**41.6**	**39.1**	98.9	66.7	0.0	**2.3**	**31.0**	100	97.2	0.0	0.0	2.8	↑↓
gentamicin	94.3	57.2	0.6	**42.2**	0.0	97.7	93.6	0.6	**5.8**	0.0	100	99.4	0.6	0.0	0.0	↑↔
tobramycin	93.2	51.2	0.0	**48.8**	0.0	97.7	94.8	0.0	**5.2**	0.0	100	98.9	0.0	**1.1**	0.0	↑↓↔
clindamycin	72.2	44.9	0.0	**11.8**	**43.3**	98.3	90.8	0.0	0.0	**9.2**	100	99.4	0.0	0.0	0.6	↑↓↔
erythromycin	81.8	77.1	0.0	0.7	**22.2**	99.4	98.3	0.0	0.0	1.7	100	99.4	0.0	0.0	0.6	↑↓↔
tetracycline	62.5	54.5	0.0	**1.8**	**43.6**	96.6	94.1	0.0	0.0	**5.9**	100	100	0.0	0.0	0.0	↑↓
minocycline	65.9	13.8	0.0	**14.7**	**71.6**	96.6	72.9	0.0	0.0	**27.1**	100	98.3	0.0	0.0	1.7	↑↓
tigecycline	65.3	27.0	0.0	**73.0**	0.0	96.6	82.4	0.6	**17.1**	0.0	100	99.4	0.6	0.0	0.0	↑↓↔
linezolid	77.3	89.7	0.0	**10.3**	0.0	97.7	100	0.0	0.0	0.0	100	100	0.0	0.0	0.0	↑
fusidic acid	64.8	40.4	0.0	**59.6**	0.0	96.6	93.5	0.0	**6.5**	0.0	100	99.4	0.0	0.6	0.0	↑↓↔
rifampicin	93.2	27.4	0.6	**7.9**	**64.0**	98.3	94.2	0.6	0.0	**5.2**	100	100	0.0	0.0	0.0	↑↓↔
trimethoprim/sulfamethoxazole	77.3	97.8	0.0	0.7	1.5	97.2	97.1	0.0	**2.3**	0.6	100	99.4	0.0	0.0	0.6	↑↓↔
average	82.2	48.7	0.1	**33.4**	**17.8**	97.9	88.8	0.1	**5.8**	**5.3**	100	99.0	0.1	0.5	0.4	
S. epidermidis, $n = 289$																
cefoxitin	16.6	58.3	0.0	**41.7**	0.0	67.1	83.5	0.5	**16.0**	0.0	100	99.7	0.3	0.0	0.0	↑↓
norfloxacin	23.5	91.2	**1.5**	**7.4**	0.0	56.1	97.5	0.6	**1.9**	0.0	99.3	98.6	0.3	1.0	0.0	↑↓↔
ciprofloxacin	22.8	80.3	0.0	**19.7**	0.0	61.9	92.2	0.0	**7.8**	0.0	99.3	97.6	0.7	**1.7**	0.0	↑↓↔
levofloxacin	15.2	65.9	0.0	**9.1**	**25.0**	59.9	89.0	0.0	**1.7**	**9.2**	97.9	97.5	0.0	0.0	2.5	↑↓↔
gentamicin	19.7	57.9	0.0	**42.1**	0.0	67.5	81.5	0.0	**18.5**	0.0	99.7	99.7	0.0	0.3	0.0	↑↓↔
tobramycin	18.3	90.6	0.0	**9.4**	0.0	65.1	93.6	0.5	**5.9**	0.0	99.3	94.4	**4.5**	**1.0**	0.0	↑↓
clindamycin	41.2	84.0	0.0	**4.2**	**11.8**	77.2	88.8	0.0	**1.8**	**9.4**	99.7	97.6	0.0	0.0	2.4	↑↓↔
erythromycin	41.2	95.0	0.0	**2.5**	2.5	73.0	96.7	0.5	**1.4**	1.4	99.0	96.5	0.7	0.0	2.8	↑↓
tetracycline	10.7	67.7	0.0	**3.2**	**29.0**	59.5	75.6	0.0	**2.3**	**22.1**	99.3	91.6	0.0	0.0	**8.4**	↑↓
minocycline	11.1	37.5	0.0	**9.4**	**53.1**	60.9	65.3	0.0	**2.8**	**31.8**	99.7	95.5	0.0	0.0	4.5	↑↓↔
tigecycline	11.1	75.0	0.0	**25.0**	0.0	61.2	94.9	0.0	**5.1**	0.0	99.7	99.7	0.0	0.3	0.0	↑
linezolid	12.8	97.3	0.0	**2.7**	0.0	63.7	100	0.0	0.0	0.0	99.7	100	0.0	0.0	0.0	↑↔
fusidic acid	11.1	81.3	0.0	**18.8**	0.0	58.5	89.9	0.0	**10.1**	0.0	99.7	99.7	0.0	0.3	0.0	↑↓↔
rifampicin	26.0	82.7	0.0	**1.3**	**16.0**	73.4	99.1	0.0	0.5	0.5	99.3	100	0.0	0.0	0.0	↑↓↔
trimethoprim/sulfamethoxazole	15.2	97.7	0.0	0.0	2.3	51.9	92.0	0.7	0.0	**7.3**	99.0	89.5	**1.0**	0.3	**9.1**	↑↓↔
average	19.8	77.5	0.1	**13.1**	**9.3**	63.8	89.3	0.2	**5.0**	**5.5**	99.4	97.2	0.5	0.3	2.0	

mEs, minor errors, MEs, major errors; vMEs, very major errors.

Readability was defined as the percentage of clinical isolate/drug combinations for which a diameter measurement after a given incubation time could be determined. vMEs and MEs with values >1 and mEs with values >5 are marked in bold. Increasing, decreasing or stable change patterns of inhibition zones over time are displayed with arrows (↑, ↓ and ↔, respectively). The dominant change patterns are marked as bold arrows.

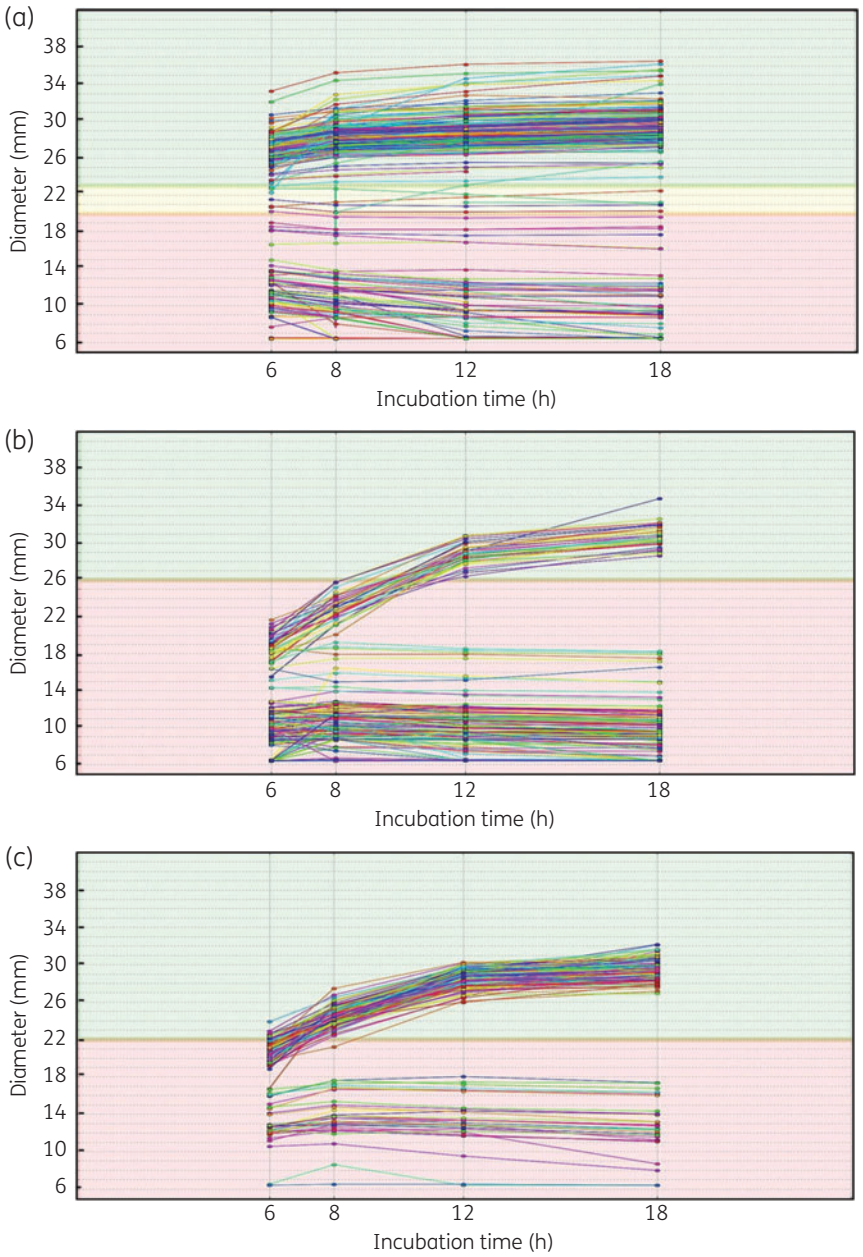

Figure 1. Diameter changes over time for selected drug/species combinations. Changes of the inhibition zone diameters over time read after 6, 8, 12 and 18 h of incubation: (a) ceftriaxone and *E. coli*; (b) penicillin G and *S. aureus*; and (c) cefoxitin and *S. aureus*. Each line represents an individual clinical isolate. The green area indicates susceptible categorization according to EUCAST 2016 CBPs, the yellow area indicates intermediate categorization and the red area reflects resistant categorization.

discriminated at early reading times, a buffer zone would be useful. Such a zone of methodological uncertainty (ZMU; e.g. for *S. aureus* and penicillin G; Figure 1b) would cover borderline isolates whose classification as either susceptible or resistant is uncertain. The definition of ZMUs could be supported by early ECOFFs defining the WT population and the resistant cut-offs (RCOFFs) delineating the non-WT populations.[30] All isolates within the ZMU, i.e. in the overlapping part of WT and non-WT populations, would be categorized as 'uncertain' and should not be reported at early reading. To define such TDCs and ZMUs, it will be necessary to test and analyse defined WT and non-WT populations and to expand rAST to other groups/genera than those contained in this work.

In summary, our study demonstrates several key findings: (i) early reading is possible for the most frequently encountered pathogens from blood cultures; (ii) precision of disc diffusion ASTs is not hampered by early reading; (iii) zone diameters change over time and may result in both major and very major errors when applying existing 18 h based CBPs of CLSI/EUCAST; (iv) patterns of

inhibition zone diameter changes are phenotype/drug combination dependent; and (v) specific expert rules and cut-offs will be necessary to allow for reliable interpretation and reporting of rAST results.

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Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online.

References

1 Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. *Clin Microbiol Infect* 2016; **22**: 416–22.

2 Ferrer R, Martin-Loeches I, Phillips G *et al*. Empiric antibiotic treatment reduces mortality in severe sepsis and septic shock from the first hour: results from a guideline-based performance improvement program. *Crit Care Med* 2014; **42**: 1749–55.

3 Kumar A, Roberts D, Wood KE *et al*. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006; **34**: 1589–96.

4 Lautenbach E, Patel JB, Bilker WB *et al*. Extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. *Clin Infect Dis* 2001; **32**: 1162–71.

5 Blaschke AJ, Hersh AL, Beekmann SE *et al*. Unmet diagnostic needs in infectious disease. *Diagn Microbiol Infect Dis* 2015; **81**: 57–9.

6 Caliendo AM, Gilbert DN, Ginocchio CC *et al*. Better tests, better care: improved diagnostics for infectious diseases. *Clin Infect Dis* 2013; **57** Suppl 3: S139–70.

7 Fisher MA, Stamper PD, Hujer KM *et al*. Performance of the Phoenix bacterial identification system compared with disc diffusion methods for identifying extended-spectrum β-lactamase, AmpC and KPC producers. *J Med Microbiol* 2009; **58**: 774–8.

8 Lavallee C, Rouleau D, Gaudreau C *et al*. Performance of an agar dilution method and a Vitek 2 card for detection of inducible clindamycin resistance in *Staphylococcus* spp. *J Clin Microbiol* 2010; **48**: 1354–7.

9 Wiegand I, Geiss HK, Mack D *et al*. Detection of extended-spectrum β-lactamases among Enterobacteriaceae by use of semiautomated microbiology systems and manual detection procedures. *J Clin Microbiol* 2007; **45**: 1167–74.

10 EUCAST. *Report from the EUCAST Subcommittee on the Role of Whole Genome Sequencing (WGS) in Antimicrobial Susceptibility Testing of Bacteria for Consultation*. May 2016. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/2016/EUCAST_WGS_report_consultation_20160511.pdf.

11 Hrabak J, Studentova V, Walkova R *et al*. Detection of NDM-1, VIM-1, KPC, OXA-48, and OXA-162 carbapenemases by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2012; **50**: 2441–3.

12 Mirande C, Canard I, Buffet Croix Blanche S *et al*. Rapid detection of carbapenemase activity: benefits and weaknesses of MALDI-TOF MS. *Eur J Clin Microbiol Infect Dis* 2015; **34**: 2225–34.

13 Oviano M, Fernandez B, Fernandez A *et al*. Rapid detection of Enterobacteriaceae producing extended spectrum β-lactamases directly from positive blood cultures by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Clin Microbiol Infect* 2014; **20**: 1146–57.

14 Sparbier K, Lange C, Jung J *et al*. MALDI biotyper-based rapid resistance detection by stable-isotope labeling. *J Clin Microbiol* 2013; **51**: 3741–8.

15 Keller MS, Kastner KR, Wojack BR *et al*. Reproducibility of the Accelerate ID/AST blood culture assay at multiple clinical sites. Poster, *ASM General Meeting, Boston, MA, USA*, 2016. http://acceleratediagnostics.com/wp-content/uploads/2016/09/ASM-2016-Reproducibility-Study-Poster.pdf.

16 Maurer FP, Castelberg C, Quiblier C *et al*. Evaluation of carbapenemase screening and confirmation tests with Enterobacteriaceae and development of a practical diagnostic algorithm. *J Clin Microbiol* 2015; **53**: 95–104.

17 Polsfuss S, Bloemberg GV, Giger J *et al*. Practical approach for reliable detection of AmpC β-lactamase-producing Enterobacteriaceae. *J Clin Microbiol* 2011; **49**: 2798–803.

18 Polsfuss S, Bloemberg GV, Giger J *et al*. Evaluation of a diagnostic flow chart for detection and confirmation of extended spectrum β-lactamases (ESBL) in Enterobacteriaceae. *Clin Microbiol Infect* 2012; **18**: 1194–204.

19 EUCAST. *EUCAST Guidelines for Detection of Resistance Mechanisms and Specific Resistances of Clinical and/or Epidemiological Importance, Version 1.0*, 2013. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_v1.0_20131211.pdf.

20 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-sixth Informational Supplement M100-S26*. CLSI, Wayne, PA, USA, 2016.

21 EUCAST. *Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 6.0*, 2016. http://www.eucast.org/clinical_breakpoints/.

22 Croxatto A, Prod'hom G, Faverjon F *et al*. Laboratory automation in clinical bacteriology: what system to choose?. *Clin Microbiol Infect* 2016; **22**: 217–35.

23 EUCAST. *Routine and Extended Internal Quality Control for MIC Determination and Disk Diffusion as Recommended by EUCAST, Version 6.1*, 2016. http://www.eucast.org/ast_of_bacteria/qc_tables/.

24 R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing, 2015. https://www.R-project.org/.

25 Pinheiro J, Bates D, DebRoy S *et al*. *nlme: Linear and Nonlinear Mixed Effects Models*, 2015. http://CRAN.R-project.org/package=nlme.

26 Kahlmeter G. Wide variation in activity of antibiotic discs from nine manufacturers. *Clin Microbiol Infect* 2016; **22**: 211–2.

27 Hombach M, Maurer FP, Pfiffner T *et al*. Standardization of operator-dependent variables affecting precision and accuracy of the disk diffusion method for antibiotic susceptibility testing. *J Clin Microbiol* 2015; **53**: 3864–9.

28 Hombach M, Ochoa C, Maurer FP *et al*. Relative contribution of biological variation and technical variables to zone diameter variations of disc diffusion susceptibility testing. *J Antimicrob Chemother* 2016; **71**: 141–51.

29 Hombach M, Zbinden R, Böttger EC. Standardisation of disk diffusion results for antibiotic susceptibility testing using the sirscan automated zone reader. *BMC Microbiol* 2013; **13**: 225.

30 Valsesia G, Hombach M, Maurer FP *et al*. The resistant-population cutoff (RCOFF): a new concept for improved characterization of antimicrobial susceptibility patterns of non-wild-type bacterial populations. *J Clin Microbiol* 2015; **53**: 1806–11.